REMARKS/ARGUMENTS

Applicants wish to thank Examiner Forman for extending the courtesy of the telephonic interview held on April 30, 2003 with Applicants' representatives, Jeffry Mann and Todd Esker.

I. Status of the Claims

After entry of this amendment, claims 1-3, 5-25, and 28-46 are pending. Claims 47-51 have been canceled without prejudice to future prosecution. Claims 1, 29-33, and 37 have been amended. No new claims have been added. The amendments do not introduce new matter.

II. The Invention

The invention provides assays that allow for the detection of a single molecule of interest. The single molecule is either directly or indirectly labeled with two or more differently colored semiconductor nanocrystals, or quantum dots. Quantum dots produce bright and tunable fluorescence that can be readily detected. Assays based on the detection of two or more differently colored quantum dots on a single molecule of interest provide highly sensitive assays in which the fluorescence from quantum dot-bound single molecules is spatially resolved from background noise and from unbound quantum dots.

Earlier methods of detecting a target species of interest are known as "ensemble detection". Below a threshold concentration or density, ensemble detection methods saturate, and are unable to differentiate the total signal of quantum dot-bound targets from the background noise and from unbound quantum dots (page 16, lines 10-16). Thus, the signal detected in ensemble detection is the total emission intensity for a population of a target species collected over an entire assay region. Following detection, the detected signal is compared with the emission intensities of known target species concentrations or densities, in order to quantify the amount of a target species of interest.

Single molecule counting is a departure from these earlier methods of quantifying the amount of the target species of interest. Single molecule counting is performed at concentrations or densities where individual molecules can be spatially resolved from

background noise. Thus, the signal detected is the actual, directly observed signal of quantum dots attached to an individual molecule of the target species of interest. While ensemble detection measures the total emission intensity of a population, single molecule counting involves counting optical characteristics produced from quantum dot-bound individual molecules. Because it is based upon direct observation, single molecule counting does not require the extra comparison step necessary for ensemble detection. Therefore, single molecule counting provides a less complicated and more sensitive method of quantifying the amount of a target species of interest than ensemble detection.

III. Support for the Amendments

Support for the amendments to the claims can be found throughout the specification, the drawings, and the claims as originally drafted.

During the April 30th telephonic interview, the Examiner suggested that adding the resolving step limitations of claims 47-51 to the Applicants' independent claims would add to the strength of Applicants' patentability arguments. Applicants have taken the Examiner's advice and amended claims 1, 29-33, and 37 to include limitations from claims 47-51. Support for these amendments are found on page 23, lines 19-28, as well as in claims 47-51 as filed. Applicants thank the Examiner for her guidance.

Claims 1, 29-33, and 37 have also been amended to substitute the word "copy" with the word "molecule". The present invention discloses methods for increasing the sensitivity, specificity and dynamic range of assay systems based upon the capture of a "target species" with an affinity moiety. (p. 13, lines 1-4). "Target species" can refer to the counting of an individual "copy", in a method referred to as "single target counting". (p. 14, lines 29-30). In "single target counting", the detected signal represents an individually bound target "molecule" in those instances where the target "molecule" is separated from another target "molecule" by a distance which allows for proper resolution (p. 16, lines 3-5). "Single target counting", often referred to as "single copy counting" in this application (p. 16, line 8; p. 17, line 8; p. 17, line 13), is contrasted with ensemble detection (p. 16, line 28). In ensemble detection, the detected signal represents the average emission intensity of a population of molecules (p. 15, line 31 to p.

16, line 2). Because "single copy counting" and "single target counting" are interchangeably defined as counting an individual copy and representing an individually bound target molecule, the words "copy" and "molecule" have similar meanings in the application. Therefore, exchanging "molecule" for "copy" in claims 1, 29-33, and 37 is supported by the specification.

Therefore, no new matter is introduced with this amendment.

IV. Responses to the Claim Rejections

Under 35 U.S.C. § 112, Second Paragraph

Claims 1-28, 31, 40, 41, 43 and 47 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention. Applicants respectfully traverse this rejection.

a) The method claims are clear

The Examiner has rejected claims 1-28, 40, 41 and 47 as indefinite because the claims do not recite a method step of counting. Applicants have incorporated a method step of counting into independent claim 1, and subsequently in claims 2-28, 40, and 41, which are dependent from claim 1. In addition, Applicants have canceled claim 47. Therefore, Applicants respectfully request withdrawal of the rejection.

b) There is an antecedent basis for the recitation of "said first affinity moiety" in claim 15

The Examiner has rejected claim 15 for lacking an antecedent basis for the recitation of "said first affinity moiety" in claim 15. Claim 15 is dependent from claim 1, which Applicants have amended to recite a "first affinity moiety". Since there is now an antecedent basis for the recitation of "said first affinity moiety" in claim 15, Applicants respectfully request withdrawal of the rejection.

c) There is an antecedent basis for the recitation of "a first quantum dot" and "a second quantum dot" in claims 31 and 43

The Examiner has rejected claims 31 and 43 for lacking an antecedent basis for the recitation of "said first quantum dot" and "said second quantum dot" in claim 31. Applicants

have amended claim 31 to recite "a first quantum dot" and "a second quantum dot". Claim 43 is dependent from claim 31, and thus also contains this amendment. Since there is now an antecedent basis for the recitation of "a first quantum dot" and "a second quantum dot" in claims 31 and 43, Applicants respectfully request withdrawal of the rejection.

Under 35 U.S.C. § 102(e)

To maintain a *prima facie* case of anticipation, the Examiner must demonstrate that each and every element as set forth in the claim is either expressly found or is inherently described in a single prior art reference. The identical invention must be shown in as complete detail as is contained in the ...claim. See MPEP § 2131. Applicants submit that each element of the claims now pending has not been identified in the art presently of record. Therefore, Applicants respectfully traverse the following rejections.

a) Over Bruchez, et al. ("Bruchez")

Claims 1-3, 5-18, 23, and 29-30 are rejected as allegedly being anticipated by Bruchez (U.S. Pat. No. 6,274,323). The Examiner has cited Bruchez for disclosing a method of counting the presence of a single copy of a target species in a sample. However, the cited reference fails to teach all of the claimed elements of the invention. In particular, Bruchez teaches ensemble detection.

Both Bruchez, in Example 1, and Applicants, on page 17, line 22, have subjected their inventions to a Qdot Immunosorbent Assay (QISA). In this assay, different amounts of biotinylated rabbit IgG were placed on a surface. The surfaces were washed with streptavidin functionalized quantum dots. Some of these streptavidin functionalized quantum dots bound to the biotinylated rabbit IgG. After the non-bound streptavidin functionalized quantum dots were washed away, the surfaces were imaged. In Applicants' experiment, 10 nM to 100 fM solutions of biotinylated rabbit IgG were applied to the surface. As can be seen in Figure 3A, signal from quantum dot-bound single molecules was observed. As can be seen from the ordinate of the chart in Figure 3B, these single molecules were counted and their densities recorded across the observable range.

A similar experiment is disclosed in Bruchez, however, the results are in contrast with Applicants' results. In Bruchez's Figure 2, the ordinate displays the total emission (fluorescence) intensity of a population of the quantum dot-bound target species, which is a hallmark of ensemble detection, as explained in **The Invention** section above. Therefore, Bruchez does not teach each element of claims 1-3, 5-18, 23, and 29-30 and Applicants respectfully request withdrawal of the rejection.

Additionally, Applicants have incorporated the limitations of claims 47 and 48 into independent claims 1, 29, and 30, and subsequently on claims 2-3, 5-18, and 23, which are dependent from claim 1. Because Bruchez teaches ensemble detection, Bruchez does not disclose each and every element of claims 1-3, 5-18, 23, and 29-30. Accordingly, claims 1-3, 5-18, 23, and 29-30 are in condition for allowance and Applicants respectfully request withdrawal of the rejection.

b) Over Weiss ("Weiss")

Claims 1, 3, 5-8, 10-13, 41 and 42 are rejected as allegedly being anticipated by Weiss (U.S. Pat. No. 6,207,392). The Examiner has cited Weiss for disclosing a method of counting the presence of a single copy of a target species in a sample. Applicants have incorporated the limitations of claims 47 and 48 into independent claims 1 and 29, and subsequently on claims 3, 5-8, 10-13, 41 and 42, which are dependent from claim 1. Because Weiss does not teach the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, Weiss does not disclose each and every element of claims 1, 3, 5-8, 10-13, 41 and 42. Accordingly, claims 1, 3, 5-8, 10-13, 41 and 42 are in condition for allowance and Applicants respectfully request withdrawal of the rejection.

Under 35 U.S.C. § 103(a)

In order to establish a *prima facie* case of obviousness, the rejection must demonstrate that (1) the cited references teach all the claimed elements; (2) there is a suggestion or motivation in the prior art to modify or combine the reference teachings; and (3) there is a reasonable expectation of success. MPEP § 2143; *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir.

1991). As explained below, the cited references fail to disclose all the elements of the claimed invention and fail to provide a basis for one of skill to either combine the references or reasonably expect that the references' methods would be useful for single molecule detection. Therefore, Applicants respectfully traverse the following rejections.

a) Over Bawendi et al. ("Bawendi") in view of Singer et al. ("Singer")

Claims 1-3, 5-7, 9-18, 29, 40-42, 47 and 48 are rejected as allegedly being obvious over Bawendi (U.S. Patent No. 6,306,610) in view of Singer (U.S. Patent No. 5,866,331).

The Examiner has cited Bawendi for teaching a method of detecting the fluorescence of two distinguishable quantum dots attached to a target species immobilized on a substrate. The Examiner acknowledges that Bawendi differs from the claimed invention in that Bawendi does not teach single molecule counting. Singer is cited by the Examiner for allegedly teaching single copy counting.

(1) The cited references fail to teach all the claimed elements

The references cited by the Examiner fail to teach all of the claimed elements of the invention. In particular, none of the cited references teach the counting of quantum dot-bound single molecules.

Singer claims to detect a single probe bound to a target molecule. (Column 4, lines 45-49; Abstract, last sentence.) To accomplish this, however, Singer must attach at least five fluorochromes to each probe (Column 4, lines 49-51). In order to quantify the amount of the target species, Singer must: a) measure the Total Fluorescence Intensity (TFI) of all the fluorochromes (Column 6, lines 58-62); b) know the TFI per fluorochrome (Claim 1, part e)); and c) know the TFI per probe (Claim 1, part f)). Thus, Singer is detecting the intensity of a population of target species molecules, rather than counting an optical characteristic from quantum dot-bound individual molecules, as described in Applicants' invention.

Since neither Singer nor Bawendi teach the counting of quantum dot-bound individual molecules, the references fail to teach all of the claimed elements of the invention. In

the absence of a disclosure or suggestion of each claimed element, a *prima facie* case of obviousness cannot be set forth.

(2) There is no suggestion or motivation to modify or combine the reference teachings

The references cited by the Examiner also fail to provide a suggestion or motivation to modify or combine the references. In particular, the two cited references teach the use of different numbers of fluorochromes. See Figure 1 for a comparison of the fluorochrome requirements of Bawendi and Singer. In Bawendi's method described above, two probes, each containing one quantum dot, are attached to a target species. Singer, on the other hand, requires at least five fluorochromes attached to each probe. In order to use Bawendi's two probe technique in concert with Singer's method, one would need to use no less than ten quantum dots. Since the two cited references provide conflicting information on their fluorochrome requirements, there is no motivation to combine the two. Thus, a *prima facie* case of obviousness cannot be set forth.

(3) The cited references do not provide a reasonable expectation of success

The references cited by the Examiner fail to provide a reasonable expectation of success in performing the Applicants' invention. As mentioned earlier, both Bawendi and Singer employ ensemble detection, which can involve conducting experiments at concentration or density ranges where individual molecules cannot be imaged. Since single molecule counting is very unlikely to occur at concentration or density ranges suitable for ensemble detection, Bawendi and Singer do not provide a reasonable expectation of success in performing the Applicants' method. Thus, a *prima facie* case of obviousness cannot be set forth.

The cited references teach away from the Applicants' invention

In addition to not meeting the criteria of the *prima facie* case, the cited references also teach away from the Applicants' invention. (See *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988) stating, "...[it is] error to find obviousness where references 'diverge from and teach away from the invention at hand.'" quoting *W.L. Gore and Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1550, 220 USPQ 303,311 (Fed. Cir. 1983)).

In Column 5, lines 49-57, Singer reports that,

> Despite the enhanced contrast and resolution of a restored image, **there is no way to determine the number of probes responsible for a particular point of fluorescence, unless the TFI of a single probe--in the imaging environment--is known.** The present invention provides a method of determining the TFI per fluorochrome (or per probe bearing a known number of fluorochromes) in an imaging environment. [emphasis added]

According to Singer, it is not possible to quantify the amount of a target species without knowing the TFI. However, Applicants' method is employed at low enough concentrations or densities that each point of fluorescence corresponds to a quantum dot-bound single molecule. This alleviates the need to know the TFI. Since Singer instructs that TFI must be known in order to quantify the amount of a target species, and Applicants have discovered a method that does not require this knowledge, Singer, in fact, teaches away from the Applicants' invention.

Because the cited references fail to teach all the claimed elements, do not contain a suggestion or motivation to modify or combine the reference teachings, and do not provide a reasonable expectation of success, a *prima facie* case of obviousness cannot be set forth. In fact, the cited references specifically teach away from the methods in Applicants' invention. Thus, Applicants respectfully request withdrawal of the rejection.

b) Over Bawendi in view of Singer and Barbera-Guillem et al. ("Barbera-Guillem")

Claim 8 is rejected as allegedly being obvious over Bawendi and Singer as applied to claim 7 in part a) above and further in view of Barbera-Guillem (U.S. Patent No. 6,309,701).

Bawendi and Singer are discussed above. The Examiner has cited Barbera-Guillem for teaching a method of detecting a target species with first and second quantum dots with distinct emission spectra which combine to form a third color whereby targets present in minute quantities are detectable and multiple targets are distinguishable in multidimensional formats. The references cited by the Examiner fail to teach all of the claimed elements of the

invention. In particular, none of the cited references teach single molecule counting and resolution.

1) The element of single molecule counting is not taught

As mentioned above, neither Bawendi nor Singer teach the single molecule counting element of the Applicants' invention. Likewise, Barbera-Guillem primarily describes the physical parameters and syntheses of various fluorescent nanocrystal-labeled microspheres. The only discussion of the use of these microspheres to detect analytes in a sample occurs in Example 6 (column 15, line 60 to column 16, line 63). When the presence of analyte is detected in Barbera-Guillem,

> the method may further comprise quantitating the amount of analyte by measuring the intensity of the fluorescence signal pattern emitted from the fluorescent microspheres bound to the analyte, and relating the intensity measured to the amount of analyte.
>
> (column 16, lines 39-43)

By measuring the total emission intensity produced by the microspheres, and then using this measurement to quantify the amount of an analyte, Barbera-Guillem is detecting a population of analyte molecules, otherwise known as ensemble detection. Because there is no discussion of counting quantum dot-bound single molecules, Barbera-Guillem does not teach the element of single molecule counting of Applicants' invention. As the combination of cited references does not disclose the element of single molecule counting, a *prima facie* case of obviousness cannot be set forth.

2) The element of resolution is not taught

Applicants have incorporated the limitations of claim 47 into independent claim 1, and subsequently on claim 8, which is dependent from claim 1. Because the combination of Bawendi, Singer, and Barbera-Guillem does not teach the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, the combination does not

disclose each and every element of claim 8. Thus, a *prima facie* case of obviousness cannot be set forth.

Applicants respectfully request withdrawal of this obviousness rejection.

c) Over Bawendi in view of Singer and Walt et al. ("Walt")

Claims 19-23 are rejected as allegedly being obvious over Bawendi and Singer as applied to claim 1 in part a) above and further in view of Walt (U.S. Patent No. 6,327,410).

Bawendi and Singer are described above. The Examiner has cited Walt for teaching a method of detecting a target species immobilized on a substrate comprising detecting a single copy of said target species by detecting emitted fluorescence wherein the target is distributed upon said substrate in a random manner. The references cited by the Examiner fail to teach all of the claimed elements of the invention. In particular, none of the cited references teach single molecule counting and resolution.

1) The element of single molecule counting is not taught

As mentioned above, neither Bawendi nor Singer teach the single molecule counting element of Applicants' invention. Likewise, Walt primarily describes different varieties of substrates which can be utilized in certain fluorescence assays. There is no discussion of Applicants' single molecule counting element in Walt. As the combination of cited references does not disclose the element of single molecule counting, a *prima facie* case of obviousness cannot be set forth.

2) The element of resolution is not taught

Applicants have incorporated the limitations of claim 47 into independent claim 1, and subsequently on claims 19-23, which are dependent from claim 1. Because the combination of Bawendi, Singer, and Walt does not teach the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, the combination does not disclose each and every element of claims 19-23. Thus, a *prima facie* case of obviousness cannot be set forth.

Applicants respectfully request withdrawal of this obviousness rejection.

d) Over Bawendi and Singer

Claims 24, 25, 28, 32-39, 44-46, and 49-51 are rejected as allegedly being obvious over Bawendi and Singer as applied to claim 1 in part a).

Descriptions of, and arguments against, the combination of Bawendi and Singer have been presented in part a) above. As neither of these cited references teaches Applicants' single molecule counting element, a *prima facie* case of obviousness cannot be set forth.

Additionally, Applicants have incorporated the limitations of claim 47 into independent claim 1, and subsequently on claims 24, 25 and 28, which are dependent from claim 1. Because the combination of Bawendi and Singer does not teach the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, the combination does not disclose each and every element of claims 24, 25, and 28. Thus, a *prima facie* case of obviousness cannot be set forth.

Applicants respectfully request the withdrawal of this obviousness rejection.

e) Over Bawendi in view of Empedocles et al. ("Empedocles")

Claims 30, 31 and 43 are rejected as allegedly being obvious over Bawendi and Empedocles (Adv. Mater. (1999) **11(15)**:1243-1256).

Bawendi is described above. The Examiner has cited Empedocles for teaching the resolution of single quantum dots using a CCD-device wherein the resolution is affected by the environment.

Applicants have incorporated the resolving step limitation of claim 47 into independent claims 30 and 31, and subsequently in claim 43, which is dependent from claim 31. Because neither Bawendi nor Empedocles teaches the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to said single molecule, neither cited reference discloses each and every element of claims 30, 31, and 43. Accordingly, claims 30, 31, and 43 are in condition for allowance and Applicants respectfully request withdrawal of this obviousness rejection.

Under Obviousness-Type Double Patenting

a) Over Bruchez

Claims 1-3, 5-18, 23, 29, and 30 are rejected under the judicially created doctrine of obviousness type double patenting over claims 1-40 of Bruchez. Since the issued patent was filed prior to the pending patent application, a one-way obviousness test is appropriate. MPEP § 804(II)(B)(1)(a). Thus, to maintain a double patenting rejection under the judicially created doctrine of obviousness-type double patenting, the Examiner must set forth a proper *prima facie* case of obviousness. The three criteria for a *prima facie* case of obviousness are described above. First, as mentioned earlier, Bruchez teaches ensemble detection, and therefore does not teach all of the claimed elements of the Applicants' invention. Second, as Bruchez's method teaches ensemble detection, which is not part of the Applicants' invention, there is no motivation to use Bruchez's method in the practice of Applicants' invention. Finally, since ensemble detection is performed in concentration or density ranges where single molecule detection is very unlikely, there is no reasonable expectation of success in employing Bruchez's method in the practice of Applicant's invention. As a proper *prima facie* case of obviousness cannot be made over U.S. Patent No. 6,274,323 B1, Applicants submit that the instant rejection for double patenting is improper.

b) Provisional rejection over copending applications 09/784,645 and 09/882,193

Claims 1-3, 5-25, and 28-51 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-43 of copending Application No. 09/784,645, and over claims 1-26 of copending Application No. 09/882,193. As the rejection is provisional, none of the claims have yet been allowed. Therefore, Applicants request that the rejection be held in abeyance until one or more claims of the allegedly conflicting applications are found allowable.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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